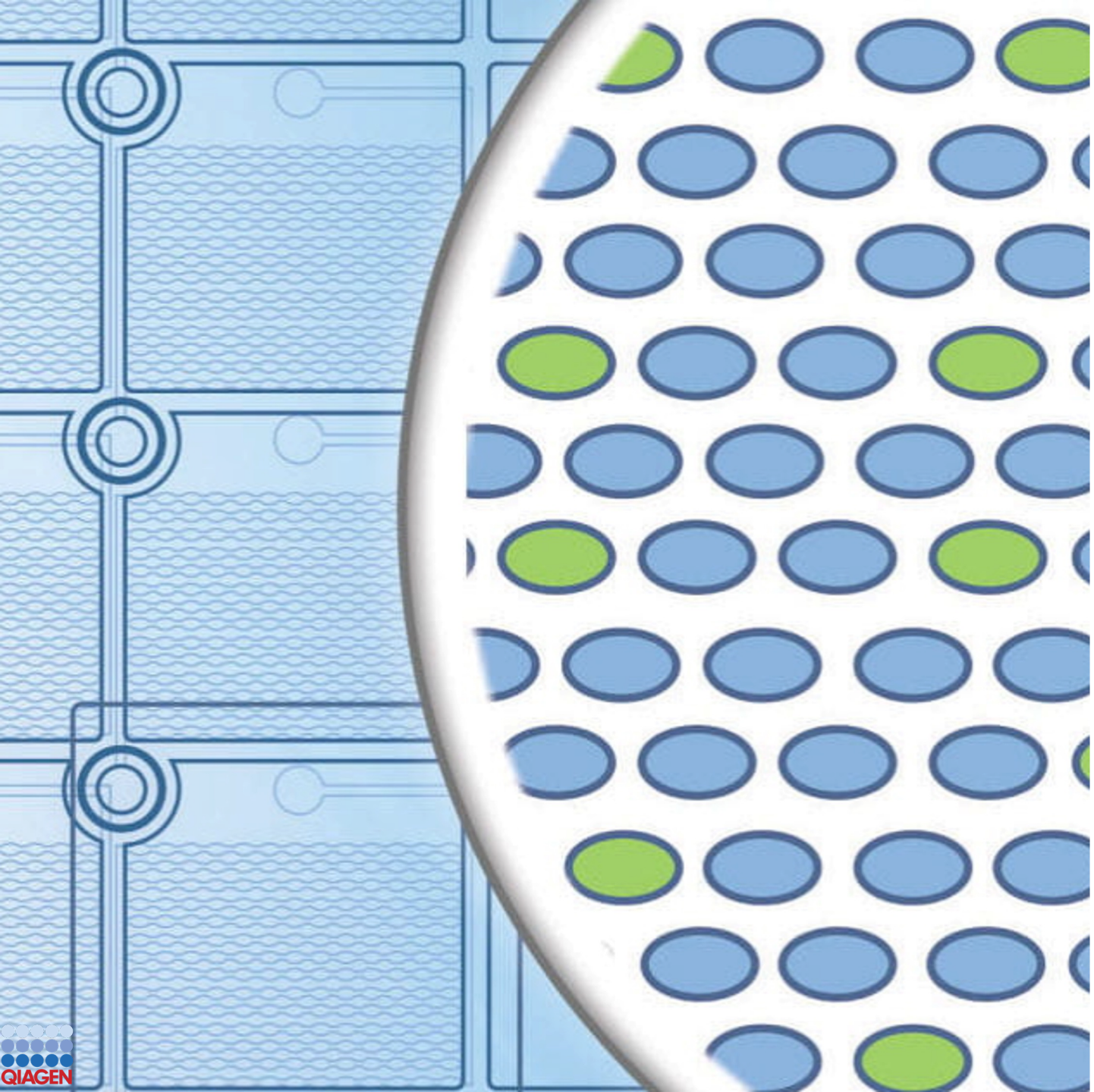


Digital PCR in NIPT

Kristína Valovičová

Iveta Valášková

Digital PCR
Applications

ONCOLOGY
- Gene amplification, CNV
- Fusions, splice variants
- Monitoring the course of therapy
- DNA methylation
- Microsatellite instability
- Minimal residual disease

RESEARCH
- Microbiom
- Gene expression, rare transcripts
- Absolute quantification
- CNV
- miRNA
- Quantification and quality control of NGS libraries
- Detection and quantification of gene editing

dPCR

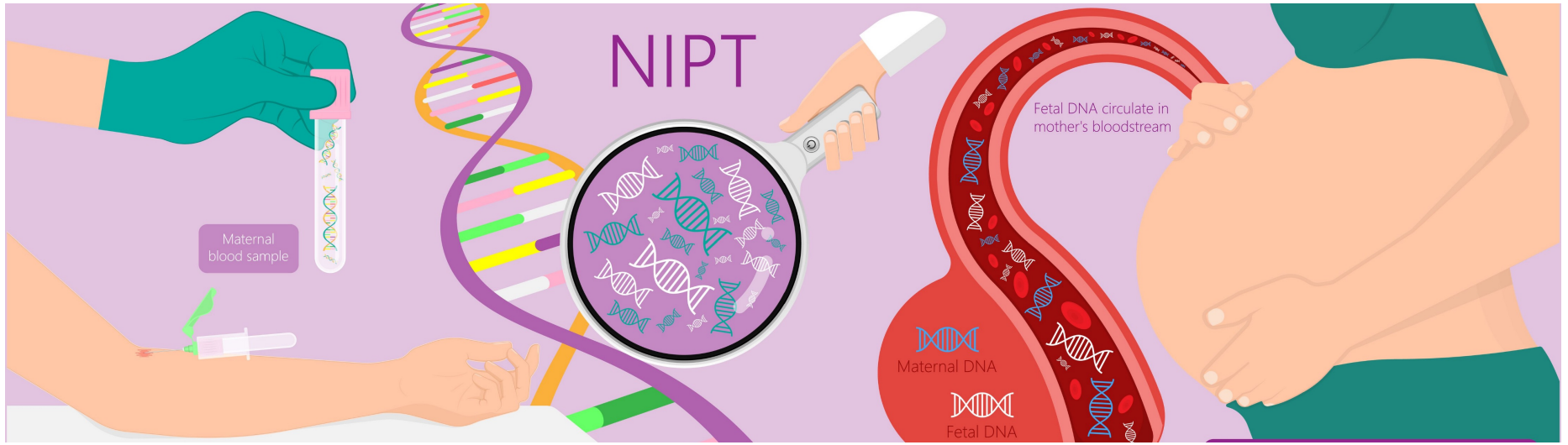
- **lower competition, higher sensitivity**
- **less effect of inhibitors**
- **higher accuracy and reproducibility**
- **easier multiplexing**
- **no standards**

PATHOGENES
- Early detection of pathogens and pathogenic mutations
- Viral load, reservoirs
- Resolution of complex samples
- Environmental monitoring
- Heteroresistance
- Food safety
- Pre/posttransplantation monitoring

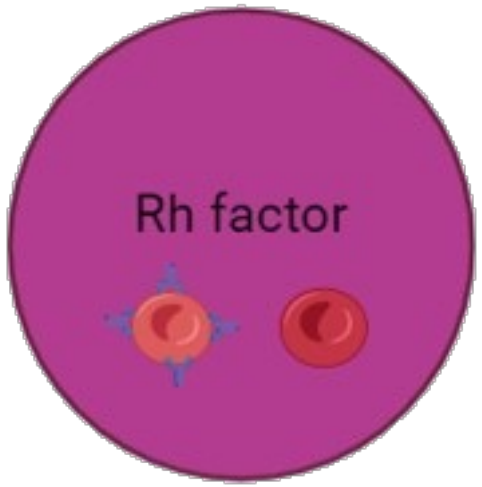
GENETICS
- Genetic, neurological, autoimmune, metabolic diseases
- mtDNA-associated diseases
- NIPT
- Graft rejection monitoring

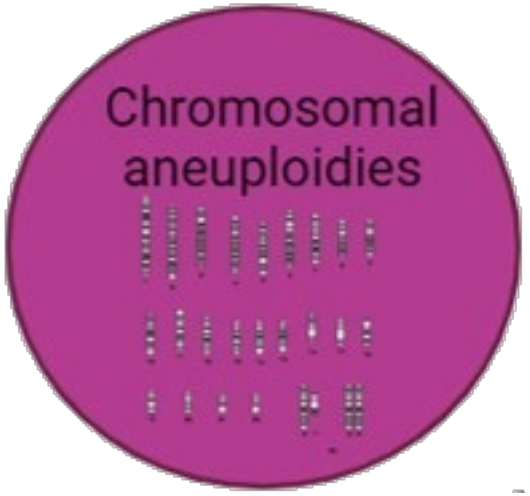
GMP, PHARMA

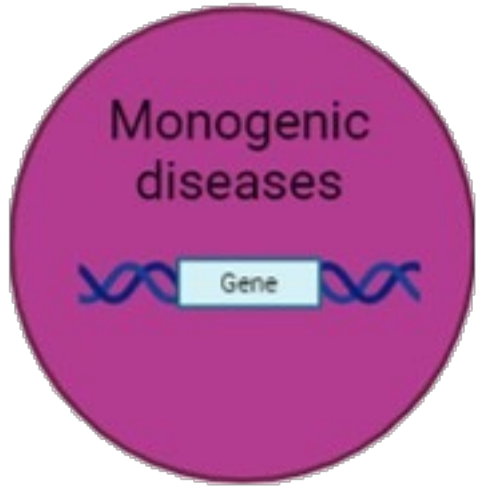
GMO


NIPT
Maternal blood sample
Fetal DNA circulate in mother's bloodstream
Maternal DNA
Fetal DNA

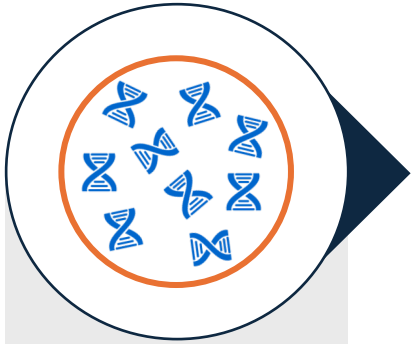

Sex determination


Rh factor


Chromosomal aneuploidies

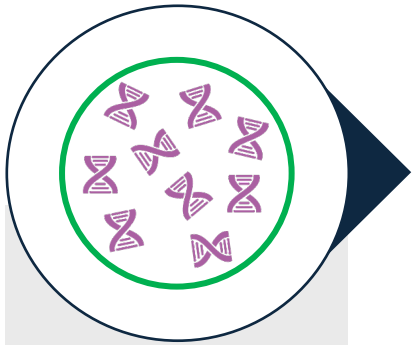

Monogenic diseases
Gene

Digital PCR
qPCR vs. dPCR



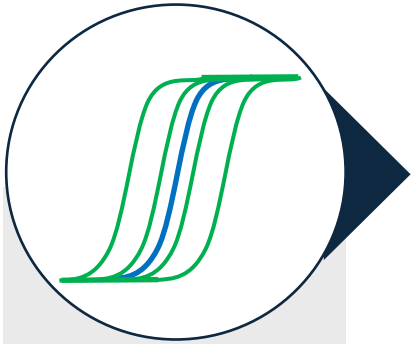
Sample reaction mixture

mastermix,primers+probes, sample



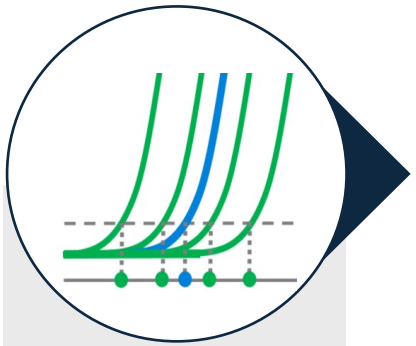
Reaction mixtures of standards

mastermix,primers+probes, standard

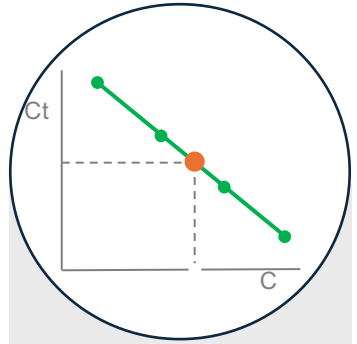


PCR detection

real-time

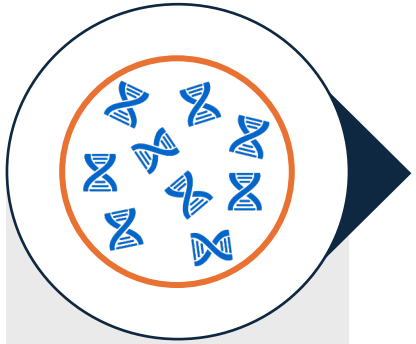


Obtaining CT values



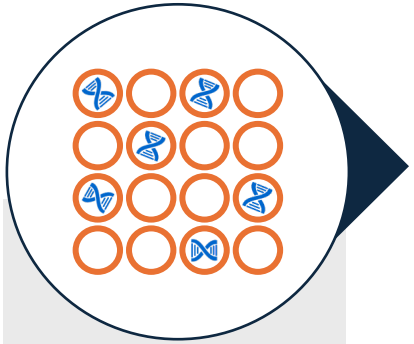
Standard curve

estimation of sample concentration by comparison with standards



Sample reaction mixture

mastermix,primers+probes, sample

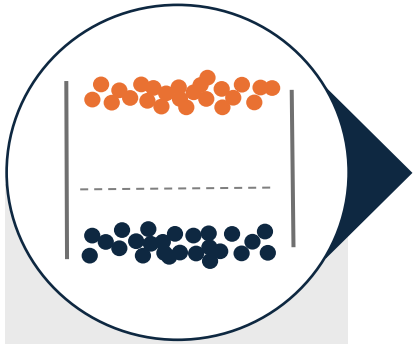


Partitioning

random distribution of NK sample copies into discrete partitions

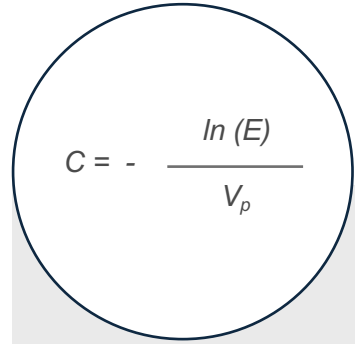
PCR detection

end-point



Clustering

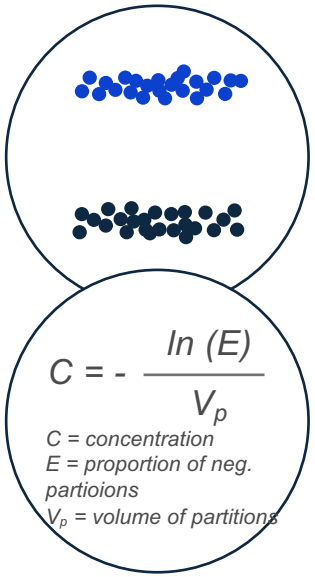
counting positive and negative reactions (partitions)

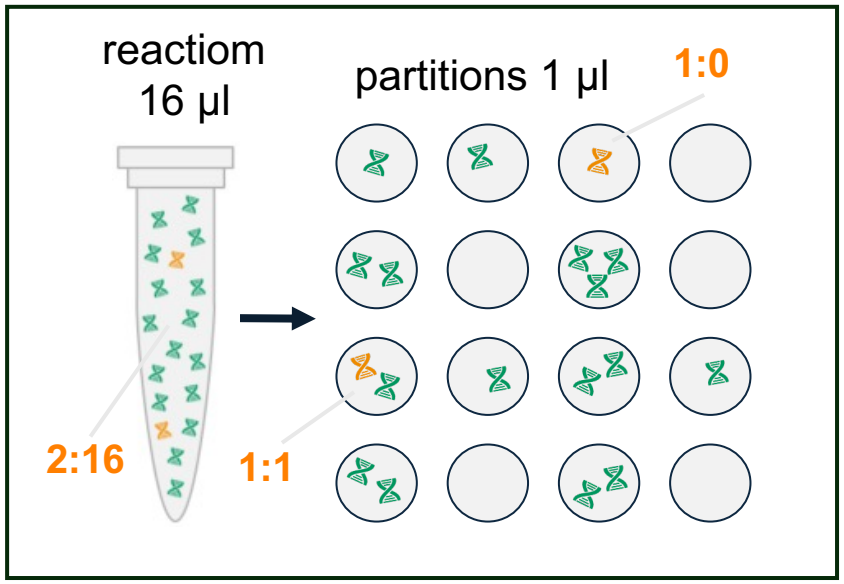


Poisson correction

estimation of sample concentration by direct molecule counting

Digital PCR
Advantages





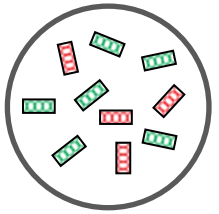
Absolute quantification without standards

direct molecular counting
PCR efficiency is not a critical factor

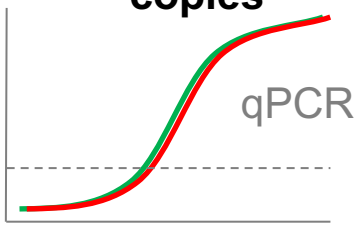
High sensitivity rare sequence detection

reduced compatibility
with wild-type sequences

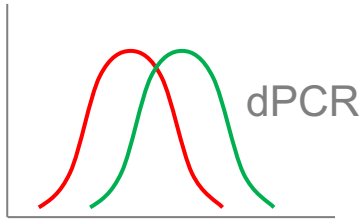
Higher volume → higher sensitivity



1000 vs. 1100 copies

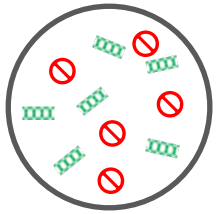


little differencein CT values

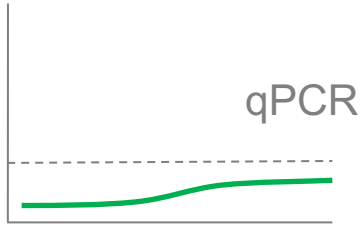


significant difference in the Poisson distribution

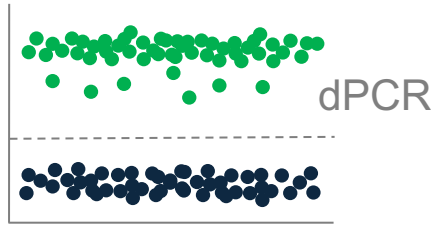
High precision



presence of inhibitors



Inhibitors reduce amplification efficiency

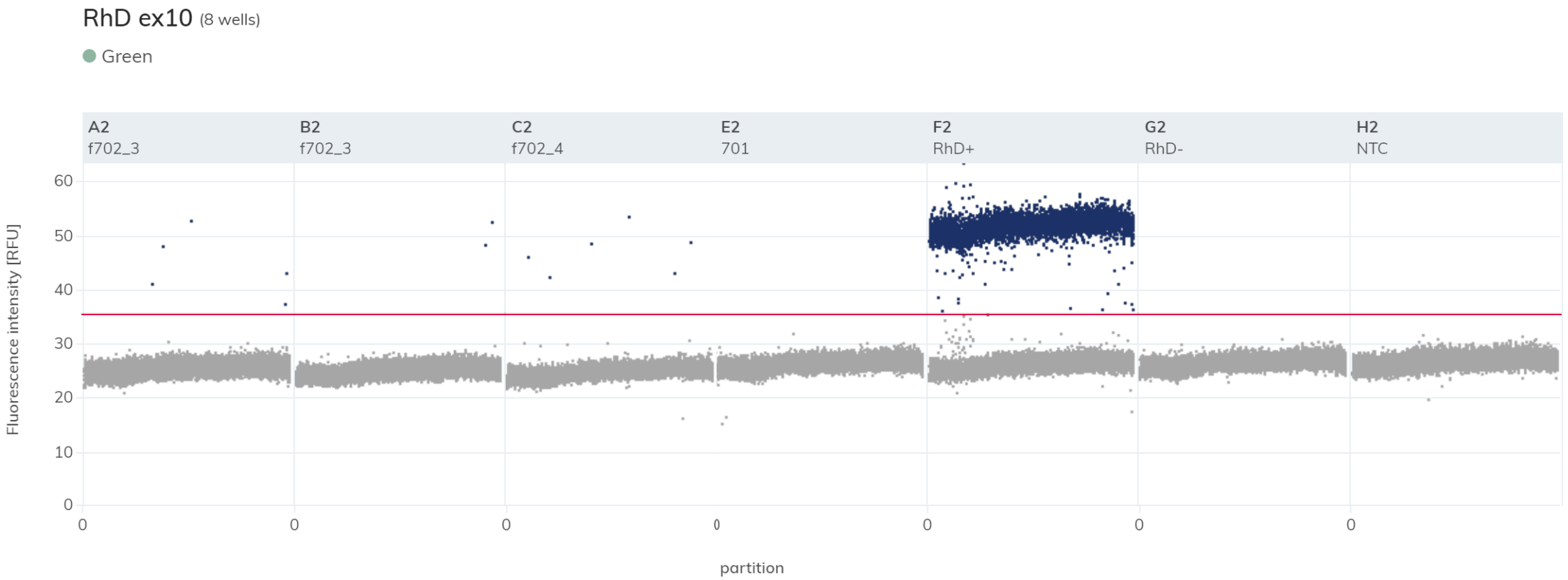


"better" target vs. inhibitor ratio, end-point detection

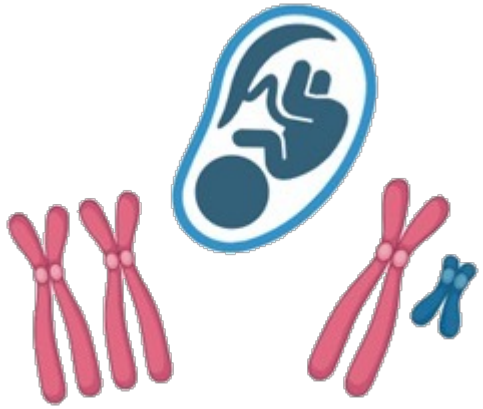
Reduced influence of inhibitors

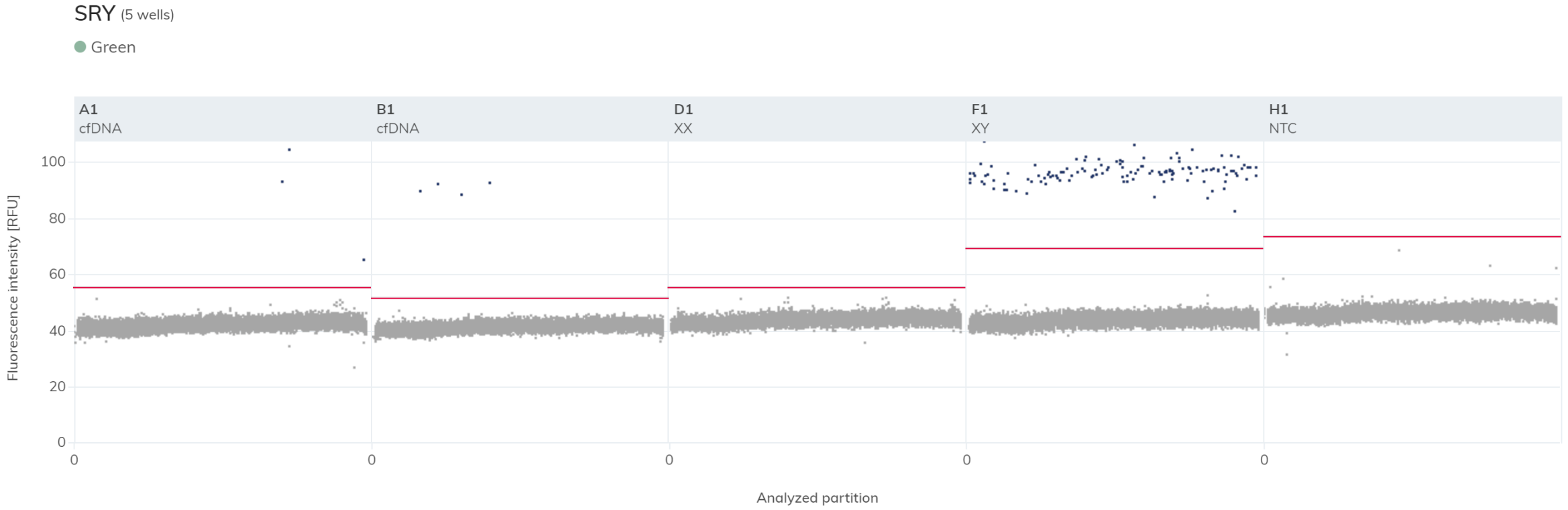
More partitions → higher precision

RhD factor


RhD ex10 (8 wells)
Green
A2
f702_3
B2
f702_3
C2
f702_4
E2
701
F2
RhD+
G2
RhD-
H2
NTC
Fluorescence intensity [RFU]
60
50
40
30
20
10
0
0
0
0
0
0
0
0
partition

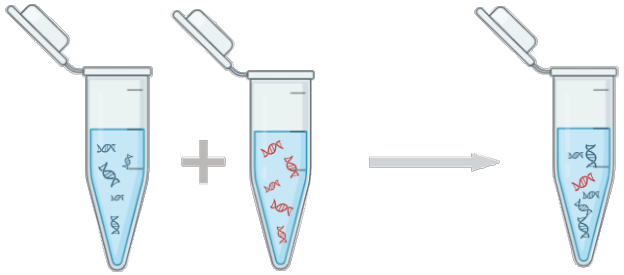
Sex determination





NIPT of achondroplasia

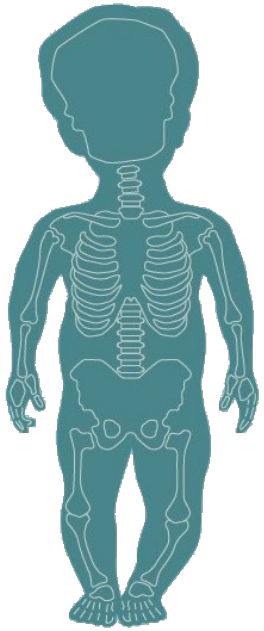
c.1138G>A/C

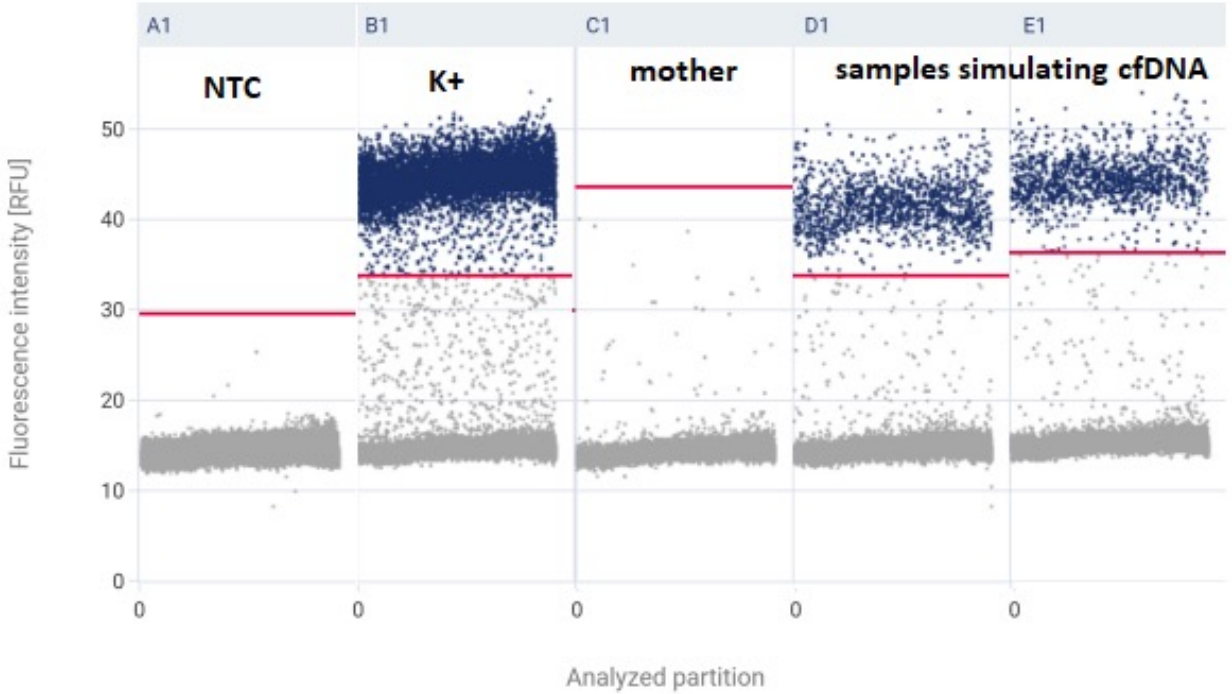


DNA of a healthy individual

DNA of a patient with confirmed achondroplasia

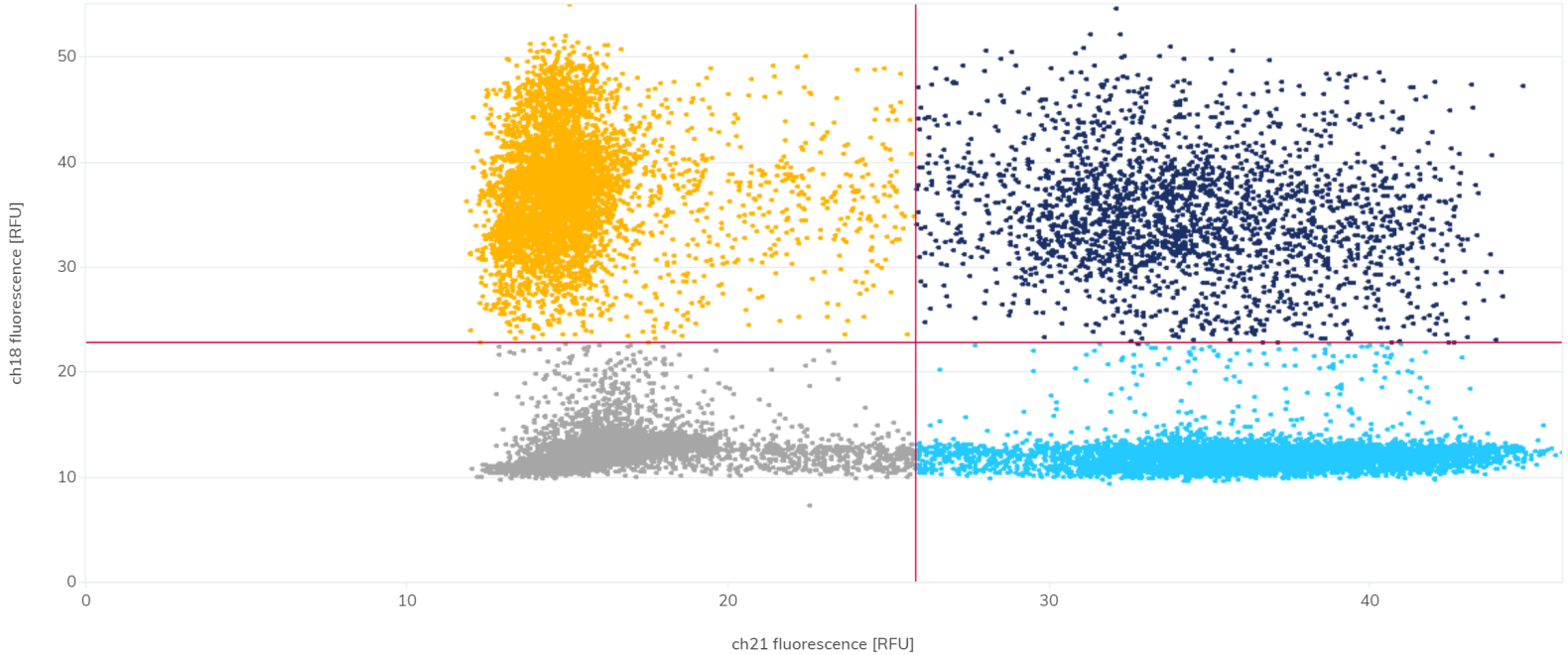
Sample simulating cfDNA (5% and 10%)





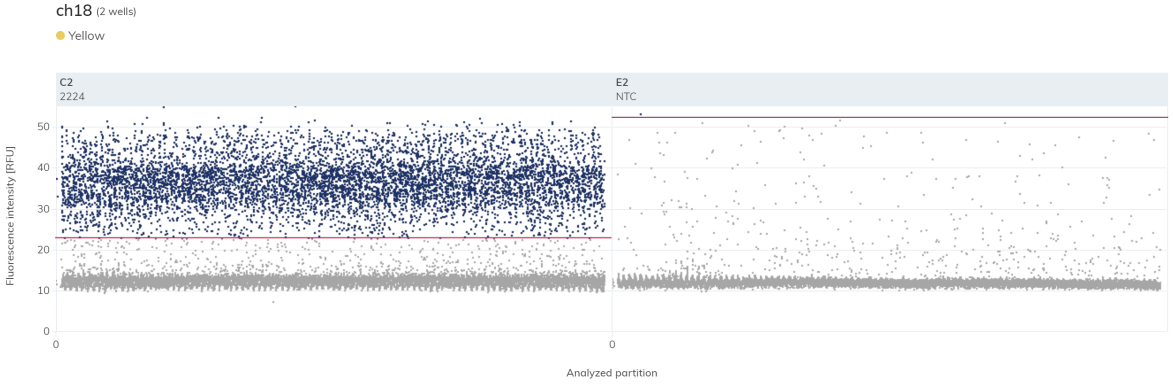
NIPS for trizomy 21 and 18

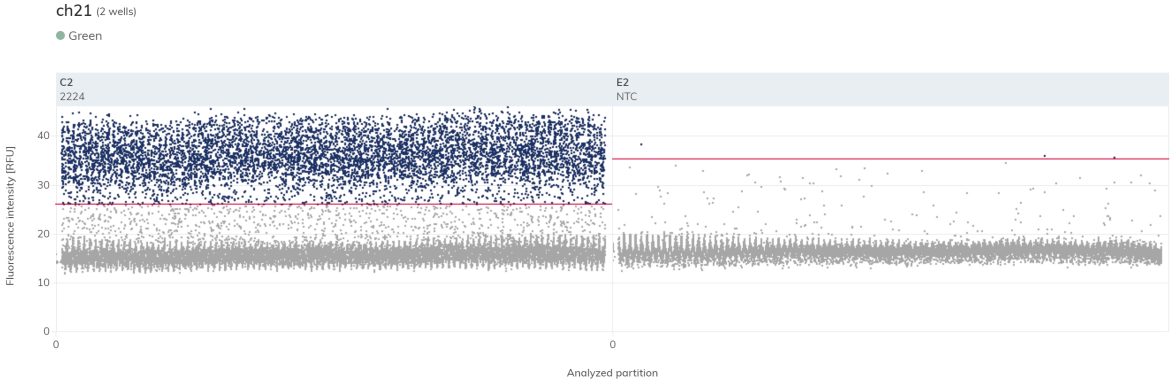
$$R_{ChrT/ChrR} = \frac{CCN\ of\ ChrT}{CCN\ of\ ChrR}$$



	X	Y	Results
	-	-	12 329
	-	+	5 128
	+	-	5 261
	+	+	2 330
Invalid on X			1 156
Invalid on Y			1 113

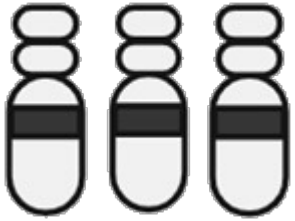
$R_{21:18}$ = 7590/7458 = **1,02**





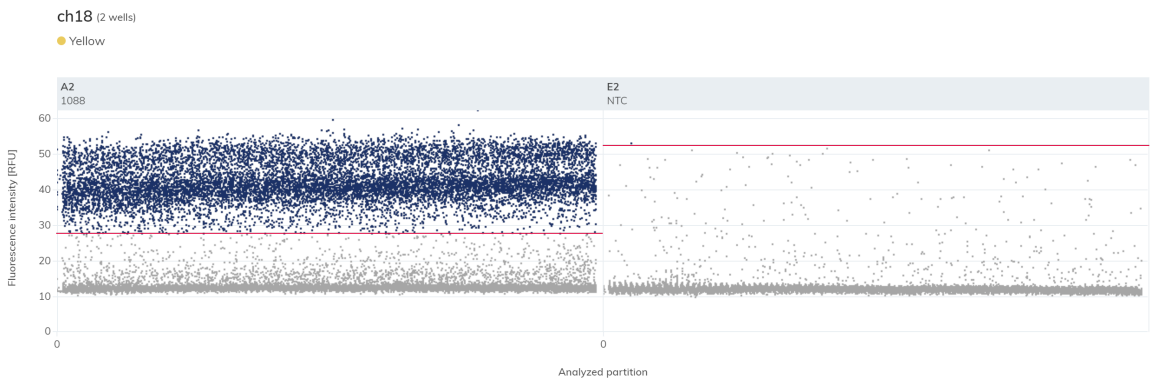
NIPS for trizomy 21 and 18

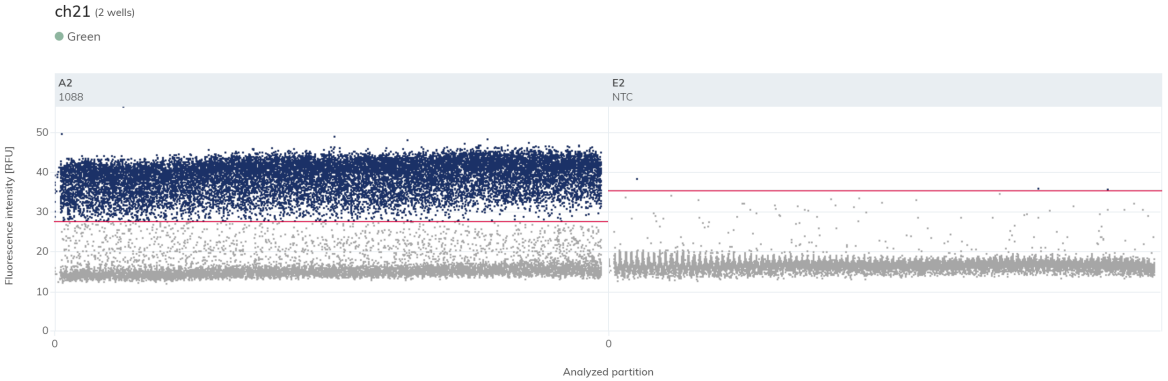
$$R_{ChrT/ChrR} = \frac{CCN\ of\ ChrT}{CCN\ of\ ChrR}$$



	X	Y	Results
●	-	-	4 328
●	-	+	4 133
●	+	-	8 697
●	+	+	8 295
Invalid on X			931
Invalid on Y			931

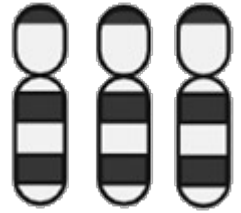
$R_{..}$ = 16992/12428 = **1,37**

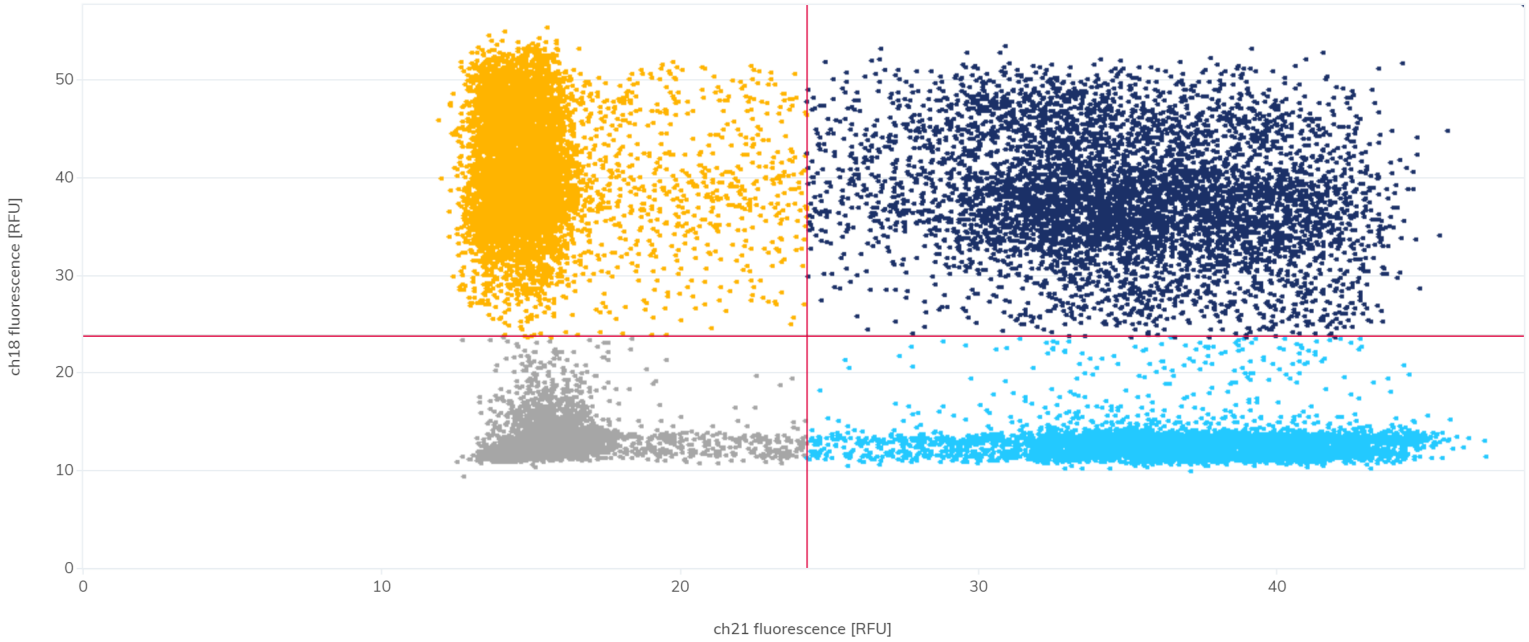




NIPS for trizomy 21 and 18

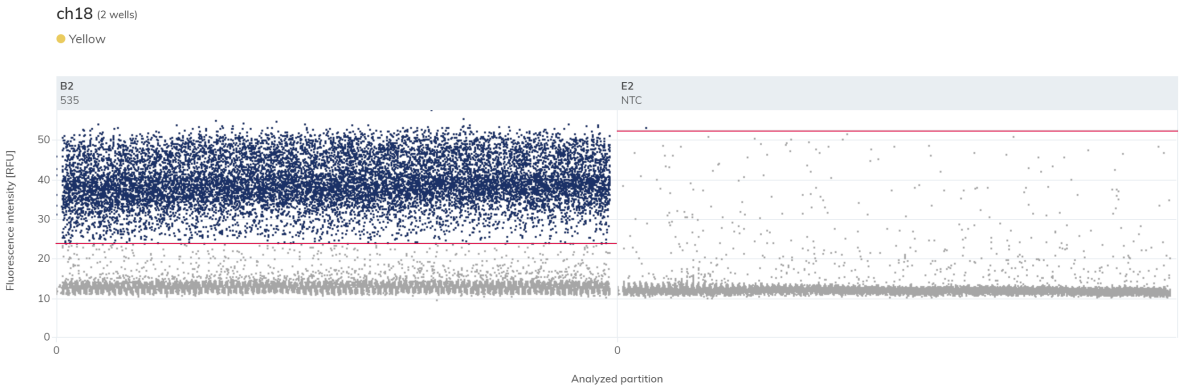
$$R_{ChrT/ChrR} = \frac{CCN\ of\ ChrT}{CCN\ of\ ChrR}$$

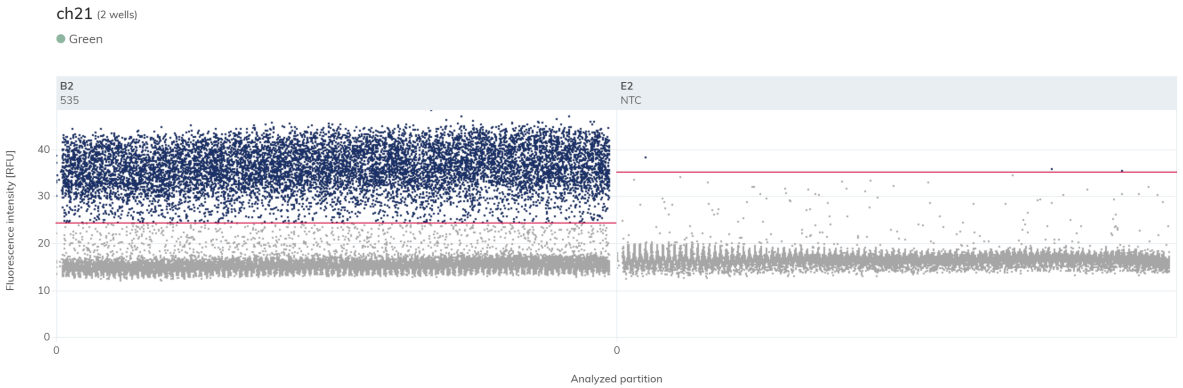




	X	Y	Results
●	-	-	6 338
●	-	+	7 872
●	+	-	5 287
●	+	+	5 961
Invalid on X			926
Invalid on Y			926

R = 13833/11248 = **1,23**





Why we chose dPCR for NIPT

- high sensitivity
- cost effective
- smaller numbers of samples
- minimum hands-on time
- turnaround time

Thanks to...

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Mgr. Zuzana Bouchalíková

Mgr. Ondřej Štěpánek, PhD

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Sophgena a.s.

FAKULTNÍ NEMOCNICE BRNO

MUNI SCI Section of Genetics and Molecular Biology

Sophgena